PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C12N 5/00, C12M 3/00
B03C 1/02, A61M 1/36
G01N 3/543, 3/553, 3/48
A61K 39/395

A1

(11) International Publication Number: WO 90/04019

(43) International Publication Date: 19 April 1990 (19.04.90)

(21) International Application Number: PCT/US89/04527

(22) International Filing Date: 10 October 1989 (10.10.89)

(30) Priority data:

255,214	11 October 1988 (11.10.88)	US
397,067	22 August 1989 (22.08.89)	US

(71) Applicant: BAXTER INTERNATIONAL INC. [US/US]; One Baxter Parkway, Deerfield, IL 60015 (US).

(72) Inventors: HARDWICK, R., Alan ; 22842 Cedarspring, El Toro, CA 92630 (US). LAKE, William, C. ; 28332 Rancho Grande, Laguna Niguel, CA 92656 (US). PRISCO, Michael, R. ; 1149 Lehnertz Circle, Aurora, IL 60505 (US).

(74) Agents: SCHIFFER, Michael, C. et al.; 2132 Michelson Drive, Irvine, CA 92715 (US).

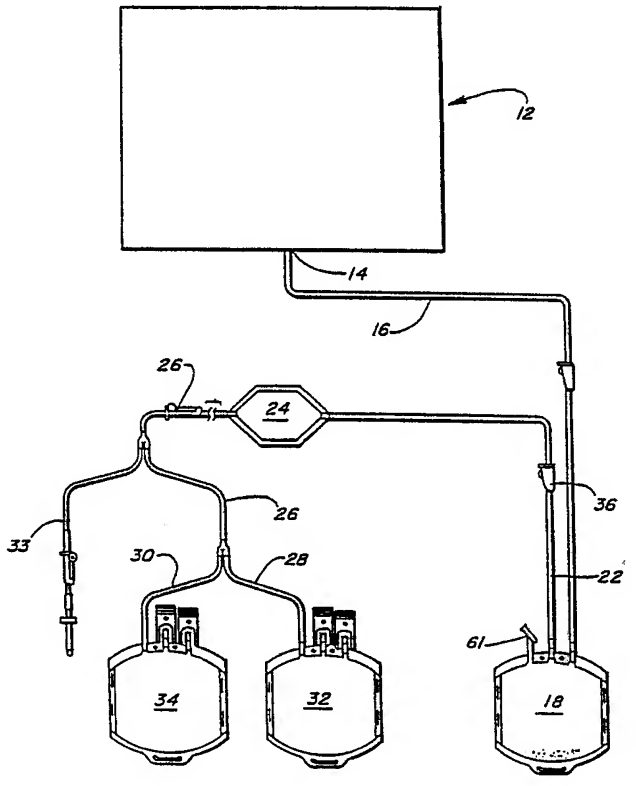
(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.
With amended claims.

(54) Title: SYSTEM FOR MAGNETIC AFFINITY CELL SEPARATION FROM CELL CONCENTRATES

(57) Abstract

A system for the selective separation of a specific cell population from a heterogeneous cell mixture is disclosed herein. Means are provided for obtaining a selective cell concentrate from the heterogeneous cell mixture by separating the selective cell concentrate based upon the physical or biological properties of the concentrate. This may be done by centrifugation, if desired. A container is provided having particle means, the particle means being attached to a substance that is active for binding to a specific desired cell. One brings the cell concentrate into contact with the particle means within the container, for example, by providing sterile connection between the site where the cell concentrate is formed and the container. This permits incubation of the cell concentrate with the particle means, to thereby selectively bind a specific cell population from the cell concentrate to the particle means, creating a particle/cell conjugate. Magnetic means are then provided for separating the particle/cell conjugate from the remainder of the cell concentrate. A two-magnetic system may be used to form the separation.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MR	Mauritania
BE	Belgium	GA	Gabon	MW	Malawi
BF	Burkina Fasso	GB	United Kingdom	NL	Netherlands
BG	Bulgaria	HU	Hungary	NO	Norway
BJ	Benin	IT	Italy	RO	Romania
BR	Brazil	JP	Japan	SD	Sudan
CA	Canada	KP	Democratic People's Republic of Korea	SE	Sweden
CF	Central African Republic			SN	Senegal
CG	Congo	KR	Republic of Korea	SU	Soviet Union
CH	Switzerland	LI	Liechtenstein	TD	Chad
CM	Cameroon	LK	Sri Lanka	TG	Togo
DE	Germany, Federal Republic of	LU	Luxembourg	US	United States of America
DK	Denmark	MC	Monaco		

System for magnetic affinity cell separation from cell concentrates.

TECHNICAL FIELD

The present invention concerns a novel system for separating a specific cell population from a heterogeneous cell mixture.

BACKGROUND OF THE INVENTION

In the field of cell separation, it is common to separate cells from plasma in blood and also to separate by centrifugation various types of cells such as red cells from white cells and the like. However, there is often a need to separate cells which are only slightly different from the other cells in a suspension thereof. If the cells are of nearly equal specific gravity, they may not be separated by centrifugation.

For example, it may be desirable to isolate various types of leukocytes from a bone marrow concentrate or a peripheral blood stem cell concentrate. It may be desirable to perform selective separation of neuroblastoma cells from a bone marrow concentrate. It may be desirable to selectively separate specific T-lymphocyte subset populations (helper-inducer or suppressor-cytotoxic T-lymphocytes) from a lymphocyte concentrate that is prepared using a blood cell separator.

Additionally, it may be desirable to selectively separate precursors of lymphokine activated killer (LAK) cells, tumor infiltration lymphocyte (TIL) cells, or activated killer monocytes, from lymphocyte or monocyte cell concentrates or from a tissue cell preparation.

By current techniques of the prior art, such as Sauer, et al, U.S. Patent No. 4,710,472, magnetic separations of individual subsets of cells from larger populations in

significant quantities become possible. This, in turn, opens up new vistas of research and therapeutic techniques, making use of the purified cell populations which may be obtained.

Another current practice in the field for cell separation utilizes hollow fiber, flat sheet membrane or packed bed bead or particle matrix materials with physically adsorbed or covalently attached chemicals or biochemicals for the selective cell separation from whole blood or the like. These devices are designed to allow continuous whole blood or blood component inflow and return. Since these devices operate at normal blood flow rates under conditions in which the concentration of desired cells can be very low compared with other cell types, the separation process is often not efficient.

DESCRIPTION OF THE INVENTION

In this invention, a system is provided for improved selective separation of a specific cell population from a heterogeneous cell mixture. The heterogenous cell mixture may first besubjected to a means for obtaining a selective cell concentrate from the heterogeneous cell mixture by separating the selective cell concentrate based upon the physical properties of the concentrate. Preferably, the selective cell concentrate is used with the system and method of the invention. Specifically, centrifugation may be used as a first separation step. There are many different types of cell centrifuge systems for the separation of desired types of cells.

A flexible, collapsible, aseptically sealed container is provided, having particle means inside, with the particle means preferably having chemically covalently attached thereto a substance capable of binding to the desired cells only, to the exclusion of other cells. Examples of such a covalently bonded substance may include antibodies, antigens, proteins, glycoproteins, polysaccharides, or lipopolysaccharides. The particle means to be in long term contact with the container walls or the like.

Means are provided for intimately contacting either the heterogenous cell mixture or the cell concentrate with the particle means within the container. This may simply be a flexible, aseptic conduit system which communicates between the site of formation of the cell mixture orconcentrate and the container which has the particle means. Incubation of the cell mixture or concentrate with the particle means may then be permitted, to cause selective binding of a specific cell population from the cell concentrate to the particles, creating a particle/cell conjugate.

Then, since the new particle/cell conjugate will have significantly different properties from the remainder of the cells, it becomes an easy matter to separate them from other cells, by centrifugation, for example. Preferably, one may use particles which are paramagnetic, then effecting separation through a magnet system.

The initial cell fluid volume may remain within the container during the incubation period, when binding to the particles takes place. Also, means may be provided for introducing additional volume of the cell mixture or concentrate into the container during the incubation period.

After separation of the particle/cell conjugate from other cells, as described above, one may separate the specific cell population from the particles or vice versa, for example by eliminating the bond between the particles and the cells in known manner, so that a purified, selected population of cells may be provided for further use. Alternatively, the unbound cells may be the desired cells, being removed from the particle/cell conjugates.

When the particles are paramagnetic, the particle/cell conjugate may be retained in a fixed location by action of the magnet means, as remaining, unbound portions of the cell concentrate are removed from the location.

Preferably, the magnet means which retains the particle/cell conjugate may define first and second spaced magnet members, one being downstream of the other, so that the second, downstream, spaced magnet member can pick up any bound particle/cell conjugate that is lost by the first magnet member, so that no particle/cell conjugate goes downstream with remaining cells.

The magnet means may be positioned adjacent to means for carrying and positioning the flexible, collapsible container which carries the particle/cell conjugate, to permit at least one inner wall portion of the container to be within the magnetic field of the magnet means. Thus, this inner wall portion serves as the fixed location at which the particle/cell conjugate is retained.

Flat-pressing means may also be provided, to press the collapsible container flat while the inner wall portion is within the magnetic field, to facilitate separation of the particle/cell conjugate from the remainder of the cell concentrate.

Specifically, the second magnet members possesses a magnetic field that is shallower and less extensive in distance than the magnetic field of the first magnet member, but stronger adjacent the magnet surface for stronger paramagnetic particle retention. Preferably, the magnetic reach of the first magnet member is substantially equivalent to the width of the fluid container being placed thereon. This ensures that the majority of the paramagnetic particles in the container will fall within the reach of the magnetic field and be drawn to the magnet surface.

It is also preferred for the container which contains the particle means to be aseptically connected to a flexible, multiple-chamber insert member for a blood cell separation centrifuge. Such an insert member may be the disposable,

blood-carrying, inner, flexible portion typically used in such a centrifuge. It may be integrally aseptically connected to the container in accordance with this invention, so that freshly collected blood cells may be aseptically transferred from the insert member to the container having the particle means, without any need of forming a sterile connection therebetween. This greatly simplifies the use in accordance with this invention, and also increases the likelihood that there is not breach of aseptic conditions.

Also, a first container having the particle means may be aseptically connected to further flexible, collapsible container means, for receiving processed cells from the first container which contains the paramagnetic particle means, or particle means of another type, if desired. Typically, a second flexible, collapsible container is sealingly, aseptically connected to and positioned between the first container described above and the further collapsible container means. This may serve as a downstream catch area which is positioned against the second magnet described above to catch any particle/cell conjugate which escapes the first magnetic means against which the first container is pressed during the cell separation operation. The second flexible, collapsible container may preferably be of hexagonal shape with inlet and outlet ports at opposed corners, to cause generally slow flow of cells through said second container relative to flow lines connected thereto. The flow line diameter is typically no more than one-fourth the width of the second container. Also the flow path, particularly through the second container during magnetic separation, should be of a very shallow depth, typically 0.02 to 0.1 inch.

Further in accordance with this invention, one may practice a method which includes the following steps. Blood from the patient may either be collected in a first container or the patient may be connected to a blood separation centrifuge,

the centrifuge being operated to form a cell concentrate which is collected in the first container. The first container is sealed. If the particle means are not already in the container, they may be placed in the container in some aseptic manner, or an inner container positioned within the container may be broken from outside of the container to cause their release within the container. Thus the cells and the particle means are mixed. Then, the primary container is connected, if not already integrally connected, to the inlet of a clamped separation set. The primary container and a desired secondary chamber between the primary container and the rest of the separation set are both placed in magnetic separator means. A primary magnet attracts the particles of the particle means, which by now are bonded to the desired cells, to retain the particle means in the primary bag as the remaining contents of the bag flow toward the separation set. The same principle takes place in the secondary chamber under the influence of a secondary magnet, to eliminate or greatly reduce the possibility of any of the particle means and attached cells flowing downstream with the rest of the contents of the primary container.

A clamp is then opened and/or a pump actuated to initiate flow from the primary container through the secondary container and across the magnetic field of the secondary magnet, into storage containers of the separation set. The storage containers may then be sealed, followed by optional disconnection of the storage containers.

Then the magnets may be removed and the adhering cells flushed, so that one or more storage containers may contain pure cell/particle conjugate, while other storage containers contain the remaining contents of the primary container. After this, a conventional process may be used to separate the particles of the particle means from their attached cells, with the cells then being separated by centrifugation or other desired means,

such as filtration or magnetic separation, and sent to a container for storage of the pure cells. The means used to break the connection between the particles and the cells depends, of course, upon the specific bonding agent. For example, the particle means may be coated with a antibody for the specific desired cells, with the result that the cells bond to the particle means. Then, when the bond is to be broken, an appropriate reagent may be used to break the bond.

An advantage of the method of this invention is use of the magnetic separating system of this invention with a cell concentrate in a batch process. In the cell concentrate the specific cell population to be separated is present at higher concentration, which tends to favor separation kinetics. Another advantage is that numerous unwanted blood cell types, in the situation where blood cells are being separated, may have already been greatly reduced in number by the preliminary, typically centrifugal cell separation process, to reduce non-specific cell reactions. For example, the collection of a lymphocyte cell concentration with minimal red blood cell, platelet, and granulocyte contamination may be effected using a blood cell separator.

Additionally, introduction of the particles of the particle means to a cell concentrate under the conditions of this invention allows incubation to take place at constant volume conditions under storage within the container having the particle means. Thus, the system solution composition can be configured to more easily obtain favorable final incubation conditions for formation of the particle/cell conjugate. These conditions may then be optimized for a specific purpose in a way which is far more versatile than otherwise.

The subsequent separation of the particle/cell conjugate is also advantageous in the conditions of this invention, with separation times being faster, and fewer cell types in the original concentrate providing a final product with fewer non-desired contaminating cells.

As previously stated, the material which is covalently attached to the particle means may be, for example, an antibody, antigen, protein, glycoprotein, polysaccharide, lipopolysaccharide. The material may also be a nucleic acid, a lipid molecule, or a synthetic or chemically modified component of such a substance which shows a selective binding affinity for the cell population to be separated. The methods used for the chemical covalent attachment of such are known and used in the production of coupled matrix material for affinity chromatography and other selective adsorption applications. Examples of such techniques of covalent attachment to sepharose, gelating, or other beads may be seen from the following articles: Habeeb, "A Novel Preparation of Immunoadsorbents," Biochimica et Biophysica Acta, 673 (1981) 527-538; Cambier, et al., "Isolated Phosphorylcholine Binding Lymphocytes. I. Use of a Cleavable Crosslinking Reagent For Solid-Phase Adsorbent Isolation of Functional Antigen Binding Cells," Journal of Immunological Methods, 51 (1982) 209-221; and Bonnafous, et al., "Ligands Immobilized Through Cleavable Mercury-Sulfur Bonds.: Journal of Immunological Methods, 58 (1983) 93-107.

The solution in which the particle means of this invention may be suspended can be a buffered sale solution which may contain a protein such as albumin, and is compatible with the physiological requirements of the heterogeneous cell concentrate and the biological binding material attached to the particle means. This solution may be configured in its chemical composition and properties to confer sterility to the substance which is covalently attached to the bead. or particle. Furthermore, the solution may be configured in its chemical composition and properties such that when a bead or particle suspension is added to the heterogeneous cell concentrate, the properties of the resulting mixture favor the formation of the bead or particle conjugate.

For example, the heterogeneous cell mixture or concentrate may be a bone marrow preparation in which the cells may be further concentrated in a cell concentrating centrifuge or the like. Additionally, the heterogeneous cell mixture can be a tissue derived cell suspension, or a cell concentrate prepared from peripheral blood using such centrifugal device. Examples of the latter are concentrates of platelets, lymphocytes, granulocyte, monocytes, or peripheral bone marrow stem cell preparations prepared with a blood cell separator such as the previously described CS3000 blood cell separator or Autopheresis-C device.

The beads or particles used in the system may be selected for particular size and specific gravity properties so as to allow the subsequent separation of the beads or particle/cel conjugate from the unconjugated cells in the heterogeneous cell concentrate using the centrifugal capabilities of a blood cell separator. A solution such as Ficoll-Hypaque or Percoll might be used to facilitate this separation.

The beads or particles of the particle means may be composed of any number of different materials such as polystyrene, latex, plastic copolymers, glass, synthetically produced gel beads and the like. Preferably, such materials will possess good mechanical properties to prevent flaking or fracturing of the beads or particles, and will allow chemical covalent attachment with ease.

It is further preferred for the beads or particles to be formed around a magnetite particle, for example, to allow separation of the bead or particle/cell conjugate using magnets, as described above. For example, particles may be produced in accordance with the methods as described in the patent application of Chaeo-huei, J. Wang, et al., Serial No. 113,294 filed October 26, 1987, entitled "Process For Producing Magnetically Responsive Polymer Particles and Applications Thereof."

The typical size of the particle means used in this invention may be from about 2 to 10 microns, preferably about 3 to 5 microns. The particles may be added in a liquid suspension, forming a typically dark sludge-like material.

On the order of 10 ml. of such liquid suspension may be placed in the bag which is to receive the cells for separation typically including one hundred thousand to 20 billion particles. In the event it is undesirable for any reason for the particles to remain in the bag for an excessively long time, due, for example, to interaction with the bag wall, they may be added separately to the bag by conventional means such as using a sterile connector, or they may reside in a frangible container within the bag, to be broken when their use is desired so that they enter the bag interior from the frangible container.

As stated above, the various containers used in this application are preferably integrally linked together in their initial manufacture so as to avoid the need for sterile connection during the processing in accordance with this invention. However, they may also be connected together with sterile connectors, numerous designs of which are well-known, for example, those of U.S. Patent No. Re. 32,056.

The Dynal Company of Great Neck, New York manufactures paramagnetic microbeads which may be used in accordance with this invention.

For a typical blood cell separation, the number of microbeads of the particle means used may number from about a hundred thousand to one billion. It has been found, when making use of a primary and secondary magnetic separator as described above, that the removal of microbeads and the cells attached to them from a cell suspension may be quantitative, with virtually no microbeads found in downstream effluent after passage through the magnetic separation system of this invention.

DESCRIPTION OF THE DRAWINGS

Figure 1 is a partially schematic plan view of apparatus in accordance with this invention for selectively separating cells.

Figure 2 is a perspective view of part of a magnetic separation device used with the apparatus of Figure 1.

Figure 3 is a perspective view of another part of magnetic separator used with the apparatus of Figure 1.

Figure 4 is a perspective view of the separation system in operating position.

Figure 5 is a plan view of a compound magnet used in the magnetic separator of Figures 2-4.

Figure 6 is an end view of the magnet of Figure 5.

Figure 7 is an end view of a second compound magnet used in the magnetic separator of Figures 2-4.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Referring to Figure 1, a disposable system 10 is disclosed for separating an individual population or populations of cells from a heterogeneous cell mixture in accordance with this invention.

Cell concentrator portion 12 which may be used withthis invention is schematically shown and may be of a design as shown in U.S. Patent Nos. 4,379,452 or 4,410,026, or may be any cell concentrator member usable in any known apparatus for the concentrating of cells. For example, the CS3000T.M. separation system sold by Baxter Healthcare Corporation may be used, or the Autopheresis-CT.M. separation system, sold also by Baxter Healthcare Corporation, or any other desired, similar device, may be used.

At outlet port 14 of concentrator system 12, a desired population of cells may be provided, for example lymphocytes, separated in concentrator system 12 from whole blood. The lymphocytes, perhaps mixed with other white blood cells, pass

through flexible conduit 16 into flexible, collapsible container 18 of generally conventional design, which contains the particle means of micron-sized particles including a paramagnetic material such as ferric oxide, coated with plastic such as polymethyl methacrylate, which, in turn, is further coated with an antibody or other cell bonding agent to the specific marker proteins in the cell wall of a given category of leukocytes, so that the leukocytes and particles 20 selectively bond to each other, to the exclusion of the remaining leukocytes and other cells.

The apparatus of this invention also carries flexible tubing 22 which communicates between flexible, collapsible container 18 and a flexible collapsible container 24 communicating with tubing 22 at one end and communicating with an outlet tubing 26 at this other end. Tubing 26 branches into tubings 28, 30 each of which connects to another flexible, collapsible container 32, 34.

While cell concentrator portion 12 is preferably integrally connected to flexible container 18, the connection may be made by a conventional sterile connector system, if desired. Additionally, the particles 20 may be stored either outside of container 18 and connected with a sterile connector system, or they may be placed in a frangible container inside of bag 28 with the container being breakable on use so that the particles 20 do not have an excessive amount of time to interact with the bag walls, in the event that some adhesion may take place there. Of course, the particles 20 may reside freely within the bag if the particles do not interact with the bag wall.

After the cells have been separated and concentrated in separation apparatus 12 and conveyed to bag 18, the primary, cell-containing container 18 may be sealed by heat sealing of line 16 and by operation of clamp 36 (although clamp 36 may be closed at an earlier stage of the operation). If the microbeads

or particles 20 are not already added to the container, they may be so added at this time, followed by gentle mixing of the contents of the primary container: specifically, the cells and the microbeads or particles. At this time, bonding takes place between the particular cells selected and the antibody coating of the particles or microbeads 20.

Following this, if container 18 is not integrally connected, as shown, with the downstream set portion of this invention beginning with chamber 24 and including bags 32, 34, such connection may be made in sterile, aseptic manner by conventional means, for example, by use of a sterile connector system of known design. In the preferred embodiment of Figure 1, the various components are integral to avoid the inconvenience of connection and the risk of contamination of the container contents.

Following this, containers 18 and 24 are placed into magnetic separator 40, which is shown in various aspects in Figures 2-7.

Separator 40 carries upper base member 42 which carries magnet assemblies 44, 46. Upper rotatable, hinged presser member 49 comprises a flat undersurface portion 50, and carried by base 42, overlying in its closed position magnet 46. Upper base member 42 thus provides two spaces, respectively against magnet 44 and magnet 46, which are respectively proportioned to receive bag 18 and bag 24. Channel 47 and space 48 provide room for tubing 22, while channel 51 is provided to receive tubing 26.

Additionally, lower base member 52 is proportioned to receive upper base member 42 in cradle area 54 so that tubing 26 can be connected to roller pump assembly 53 for controlled pumping flow of cells out of bag 18, through intermediate bag 24, and into one or the other of containers 32 or 34.

Hinged cover 56 is positioned to be brought down on top of bag 18 as installed in upper base member 42. Upper rotatable hinged presser member 49 presses down in similar manner on

flexible chamber 24. The purpose of particularly presser member 49 is to provide precise definition of the thickness of the flow path of chamber 24 during processing to cause the flow path across the associated magnet 46 to be of very shallow depth, for example, about 0.05 inch. Similarly, magnet 44 is positioned to associate with chamber 18. As will be described more fully herein, the magnets 44 and 46 are designed to provide suitably configured magnet fields to capture and retain paramagnetic particles passing through the containers positioned thereon, respectively containers 18 and 24. In this regard magnetic 44 will possess a greatermagnetic field reach than magnetic 46 in order to capture a larger percentage of particles. This places the cells passing therethrough into a position to be strongly influenced by the fields generated by magnets 44, 46. Thus, those cells bonded to magnetic beads will be retained against one or the other of the magnets.

One advantage for using a magnetic separator apparatus 40 having a separable upper base member 42 is that upper base member 42 may be refrigerated to a temperature on the order of 4° C. prior to use. Thus, the cold magnets keep the cells cold during operation, as well as providing some increase in magnetic field strength. The cold cells are less active and better preserved. Also, at low temperatures, non-specific cell interactions with the paramagnetic beads can be reduced. For example, phagocytes present are less active in ingesting available beads when kept at low temperatures.

When containers 18, 24 are lying on magnets 44, 46, clamp 36 may be opened, and pivotable pressure member 56 may be gently closed to press the container 18 flat and to press the cells and their carrier liquids out of bag 18, which rests upon magnet 44. Presser 56 may also contain magnetizable metal strips that are sized and positioned so that they are magnetically pulled to the magnet 44 in a way such that the plastic bag 18 is squeezed and the efficient flow from the bag is controlled and relatively

constant. Alternatively, the roller pump 53 may be activated to cause the cells and their carrier liquids to flow out of bag 18. As the cells and their carrier liquids flow through tubing 22, the magnetic field from magnet 44 attracts the particles or microbeads 20, and the cells to which they are bonded, causing such microbeads 20 to be generally firmly affixed against the inner wall 57 (Fig. 6) of bag 18 that is closest to magnet 44, holding the particles 20 and their attached cells as the remaining cells and suspension liquid pass through tubing 22 out of bag 18.

If any particles 20 and attached cells escape the first magnet 44, they may be caught by the interaction between container 24 and second magnet 46, upon which it lies, being retained against inner wall 59 (Fig. 7). Even under significant flow conditions, the enlarged container 24 exhibits relatively slow flow conditions through it. Hinged presser member 49 contains four metal bolts 81 that are sized to hold the presser member 49 in place with the proper spacing and mount of magnetic force. The surface 50 of presser member 49 is precisely machined to precisely define the thickness of the flow path through container 24. The shallow depth of this flow path causes any particles or microbeads present to drift into the influence of the magnetic field of magnet 46, to be retained on the inner wall 59 of container 24 as the remaining cells and liquid flow by, out of container 24, through tubing 26, into bag 32, for example. In such a circumstance, tubing 30 may be clamped off to keep bag 34 empty.

Then, when pressure member 56 has been used to squeeze all possible liquid and cells out of bag 18, a small amount of cell-compatible suspension liquid may be passed through the system, via sterile connector port 61, priming line 33, or another integrally connected container, to flush the remaining cells which are unattached to paramagnetic particles through the system into bag 32.

Then, tubing 28 may be closed, and tubing 30 opened by conventional clamp means. The system may be removed from the field of influence of magnets 44, 46, and, more suspension/solution may be passed into container 18, to flush the cells which are bound to particles 20 through tubing 22, through chamber 24, picking up any bound cells retained there, and into container 34.

Then, containers 32, 34 may be separated from the system by sealing and severing of tubings 28, 30 in conventional manner, with the desired particular population of cells being separated out from the main body of cells, and placed in separate container 34 for separate use.

As stated above, magnet 44 is designed to possess a greater magnetic field reach than magnet 46. Preferably, this magnetic field reach is at least equal to about three-quarters of the width of the container 18, and more preferably substantially equivalent to such width. This ensures that a majority of the paramagnetic particles within container 18 are captured by the magnetic field and drawn to the surface of magnet 44. Accordingly, magnet 44 will have a magnetic field reach of from about one-half to one inch, preferably, one-half to three-quaters of an inch.

Typically, magnets having greater magnetic field reaches possess lower surface field strengths. One particle magnetic assembly which provides the desired magnetic field reach, but retains substantial surface field strength will be described with reference to Figures 5 and 6. Magnet assembly 46 may be made of similar construction. Magnet assembly 44 is shown to comprise a stack of bar magnets 64 which are separated by, and in contact with, steel pole pieces 66. As a particular advantageous feature, the like poles of adjacent bar magnets 64 in the stack face each other and particular pole piece separating them. This is demonstrated by the letters "N" and "S", each of which indicate the combined north poles or south

poles of the respective magnets which are facing each other. The bar magnets 64 define long sides 68 and ends 70, and north and south poles of the bar magnets being defined along an opposed pair of long sides as shown in Figure 5. Preferably, the bar magnets are made of a high magnetism alloy of neodymium, iron and boron. In Figure 5, the face 72 of the magnet assembly shown rests in use against an outer wall of bag 18 so that the magnetic field from magnet assembly 44 passes into container 18, for retention of particles 20.

Turning to Figure 6, an end view of magnet assembly 44 is shown, with face 72 being the face that is displayed in Figure 5. As shown, magnets 64, separated by pole pieces 66, rest upon a non-magnetizable aluminum plate 74 or the like, to support the respective magnets and pole pieces.

It can also be seen that pole pieces 66 each define an angled groove 76 along parallel end faces which are spaced from bar magnets 64, and which are opposed to face 72 of the assembly 44, which face is the fixed location for retaining the particle/cell conjugate formed through the paramagnetic particles as in this invention.

The bar magnets 64, pole pieces 66, and support plate 74 may be bonded together in any conventional manner with non-magnetic cement, or making use of appropriate clamps or retention straps. Appropriate magnets for use herein may be obtained from the Crucible Magnetics Co. of Elizabethtown, Kentucky. Magnet assembly 44 contains magnets 64 that are .50 inch thick and pole pieces 66 that are .25 inch thick. This generates a magnetic field that has local maxima of 7000 to 9100 gauss (average 8300 gauss) at the surface of the magnet and which decreases to 1100 to 1700 gauss (average 1400 gauss) at a distance of 1 cm. from the magnet surface. This magnet assembly gives both a relatively strong magnetic holding force at the magnet surface and a relatively good magnetic "reach out" force to capture beads some distance (up to 1") from the magnet surface.

Magnet assembly 46 contains magnets 64 that are .25 inch thick and pole pieces 66 that are 0.1 inch thick. This generates a magnetic field that has local maxima at the magnet surface ranging from 7300 to 8000 gauss and which decreases to 80 to 500 gauss at a distance of 1 cm from the magnet surface. Compared to magnet assembly 44, magnet assembly 46 has a stronger magnetic holding force at the magnet surface, but has less magnetic "reach out" force and thus has a shallower magnetic field. The second flexible container 24 is designed to take advantage of the particular magnetic field of magnet 46. First container 18 is shown to rest on magnet assembly 44 for cell separation.

Accordingly, a system is provided for isolating in an aseptic, simplified manner a particular subclass of concentrated cells, which may be separated as shown for any desired purpose.

The following examples and the other disclosure of this application are provided for illustrative purposes only, and are not intended to limit the scope of the invention of this application, which is as described in the claims below.

EXAMPLE 1

This example illustrates a specific application of the apparatus and method of this invention in the separation of T-helper/inducer lymphocyte cells collected from whole blood.

Preparation of Mononuclear Cell Suspension:

Approximately 500 ml. of whole blood was collected into a standard Fenwal Sodium Citrate Double Blood Pack and divided between the two packs to give 290 ml. in each pack. Hespan Hetastarch (55 ml.) was added to each pack, and the contents mixed by gently tilting of the pack in a back and forth motion. The red cells were allowed to settle, and the plasma layer which contains mononuclear cells was transferred to a standard Fenwal 600 ml. transfer pack using a Fenwal plasma extractor and standard transfer tubing set. The transfer pack was centrifued

to pellet the cells from the plasma, and the plasma was transferred to another transfer pack using the plasma extractor. The cells were resuspended by adding 80 ml. of Hanks Balanced Salt Solution (HBSS) which 10 percent Fetal Calf Serum and gentle titling of the bag back and forth. An aliquot of the cell suspension was counted in 2 percent acetic acid and used to calculate a total cell harvest of 2.8×10^9 mononuclear cells for the cell suspension. After counting of the cells, an additional 100 ml. of HBSS was added to the transfer pack, and the cell suspension was mixed. One-half of the cell suspension was transferred to a second transfer pack to give a total of about 1.4×10^9 mononuclear cells in 100 ml. HBSS in each bag.

Preparation of Paramagnetic Beads
Bonded With Antibody

About 1 gram of paramagnetic beads (Pandex beads with amino surface functional groups) were washed five times with saline solution and resuspended in 20 ml. of saline in a 50 ml. glass test tube. Freshly prepared N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP) comprising 1.0 ml. of 20 millimolar solution of SPDP and absolute ethanol, was added to the test tube. The tube was rotated end-over-end for 30 minutes at 4° C. to form 3-(2-pyridyldithio)-propionyl-derivitized beads. The beads were collected by magnet and washed five times with 20 ml. aliquots of phosphate buffered saline. The beads were then suspended in 20 ml. of 50 millimolar dithiothreitol in sodium acetate (0.1 Ml buffer, pH 4.5) and incubated with end-over-end rotation for 30 minutes at 4° C. to form the thiol derivative. The bead preparation was then washed five times in phosphate buffered saline, collecting the beads with a magnet after each wash. Finally, the beads were resuspended in 20 ml. of phosphate buffered saline.

Mouse anti-Leu 3a antibody (type CD4 antibody-2mg. in 2 ml. phosphate buffered saline) was added to 0.1 ml. of 20mM SPDP in absolute ethanol. The mixture was dialyzed overnight against 500 ml. of phosphate buffered saline.

The resulting antibody solution was added to the derivitized bead suspension in a 50 ml. glass centrifuge tube. The tube was rotated end-over-end overnight at room temperature to cause coupling of the antibody to the beads. Then, the beads were washed five times with phosphate buffered saline and resuspended in 30 ml. of phosphate buffered saline.

Thus, while the created antibody carries pyridyldithio active groups, the beads, because of their dithiothreitol treatment, carry bound sulfhydryl active groups. Accordingly, upon being brought together, the antibody and the beads become chemically bonded together through a disulfide linkage resulting from a condensation reaction, with 2-thiolpyridine being split off as a by-product.

Coupling of Mononuclear Cells to
CD4 Antibody Bonded To Beads

Approximately 300 mg. of the antibody-bonded beads prepared as above (7 x 10^9 beads) were added to each bag of cell suspension prepared as previously described, using a 20 ml. syringe with attached 21 gauge needle. The resulting suspension had about a 5 to 1 bead to cell ration, and was gently mixed, being then placed on a Cole-Parmer rotator at a setting of 4 for 30 minutes at 4° C. The bag 18 (Fig. 1) containing the bead and cell suspension was connected to a 600 ml. Fenwal transfer pack 32 through tubing 22, 26 which communicates through an enlarge chamber 24, as also illustrated in Fig. 1. Bag 18 was also connected through the priming line 33 of the tubing set to a 1,000 ml. bag of physiological saline solution to allow priming of the set between container 18 and the empty transfer pack 32.

After priming the resulting interconnected bags system was installed into upper magnet tray 42 as shown particularly in Fig. 2, with bag 28 being placed in area 44, and container 24 being placed in area 46. The appropriate tubing 22 was stored in area 48, passing through channel 47, while tube 26 was installed in channel 51, and threaded through roller pump 53. The respective magnets 44, 46 were prechilled to about 4° C for continued cooling of the cells. Hinged covers 49, 56 were then closed, with the upper tray 42 installed on lower tray 52 (Fig. 4).

After about 5 minutes had elapsed, to allow complete bonding of the appropriate cells to the beads through the bonded CD4 antibodies, and to allow maximum capture of the resulting bead-cell conjugates at the inner surface of bag 18 adjacent magnet 44, roller pump 53 was actuated to pump at a rate of 10 ml. of fluid per minute into collection bag 32. Any bead-cell conjugates that escaped from bag 18 were subjected to the influence of secondary magnet 46 in bag 24, to be captured on the inner surface of chamber 24 adjacent that magnet, while cells which were not so bonded to the beads flowed out with fluids to collection bag 32.

After substantial emptying of bag 18 and container 24 of fluids, the bead-cell conjugate in bag 18 was resuspended in 100 ml. of physiological saline, while removing bag 18 and container 24 from their seating on the respective magnets, and closing flow through tubing 26 while so doing. Following this, bag 18 and container 24 were reinstalled in their positions on upper magnet tray 42, and the suspending saline solution withdrawn from bag 18 by roller pump 53, as before, through tubing 26 into collection bag 32, along with any remaining cells which were not bonded to a paramagnetic bead.

Then, bag 18 and container 24 were once again removed from the influence of the magnets, after closing off flow through tubing 26. The bead-cell conjugate in bag 18 and container 24

was resuspended in 100 ml. of physiological saline containing 25mM dithiothreitol to break the bond between cells and beads. Bag 18 and container 24 were placed on a rotator at 4° C for 20 minutes. Then, bag 18 and container 24 were connected to a 1 liter Fenwal PL269 tissue culture flask (symbolized in Fig. 1 as container 34). Following this, bag 18 and container 24 were reinstalled in upper tray 42, the hinged presser plates were closed, and the bag fluid contents were flushed into the tissue culture flask 34 while the paramagnetic bead particles remain trapped adjacent the magnets.

Thus, the free lymphocytes which are positive to the action of CD4 antibody, have been separated from other cells in the mixture, and are provided in isolated form in the tissue culture flask 34, or, if desired, a blood bag 34 as specifically shown in Fig. 1.

Residual cells in the separation set downstream from bag 24 are also flushed into flask or bag 34 with more physiological saline.

The cells in flask 34 may be collected by centrifugation and resuspended in 100 ml. RPMI 1640 tissue culture medium containing supplemental glutamine and placed in a carbon dioxide incubator for subsequent further study.

EXAMPLE 2

By means of a process similar to that disclosed in Example 1, there may be removed from bone marrow cells generally, cells of the following types: B-lymphoma, neuroblastoma, breast cancer, or leukemia cells, when such cells express a tumor associated antigen on their surface recognizable by a biological attached to the bead particle surface. Thus, the invention may be used as a process for purifying bone marrow cells prior to autologous bone marrow transplant.

The bone marrow may be processed through a conventional cell separator to extract a mononuclear cell preparation

therefrom. This mononuclear cell preparation may be incubated with a panel of mouse-derived monoclonal antibodies against the tumor cells, washed to remove excess antibody, and then incubated with paramagnetic beads as described in Example 1, coated with goat antimouse antibody to selectively bind the tumor cell to the bead. The tumor cell is then removed as in Example 1, using a magnetic separator of the type disclosed.

EXAMPLE 3

As an alternative technique of the use of this invention, T-lymphocytes may be removed from the mononuclear cell preparation described in Example 2 before an allogenic transplant to prevent Graft vs. Host Disease. This may be used in the case where bone marrow grafts are provided for the treatment of cancer, or for reconstitution of the bone marrow after exposure to radiation apart from cancer treatment. In this case, the Mononuclear cell preparation from the bone marrow is treated with a mouse monoclonal antibody (for example, anti CD3) to tag the T-lymphocyte cells for binding by the goat antimouse antibody coated beads.

EXAMPLE 4

The mononuclear cell preparation previously prepared from bone marrow as in Example 2 may also be treated in a manner similar to that described above, making use of a mouse monoclonal antibody bonded to the paramagnetic beads to allow the selection of pluripotent stem cells from the mononuclear cell preparation. The isolated stem cells can thus be used for bone marrow transplant. This approach could be used to provide for autologous bone marrow transplants which are substantially free of tumor cells, while suppressing Graft vs. Host Disease. Such isolated stem cells could also be used for gene therapy, in which genes are inserted into the stem cells prior to their implantation as a bone marrow transplant.

EXAMPLE 5

By a process substantially similar to the process of Example 1, making use of an appropriate monoclonal antibody, liver hepatocytes or insulin secreting pancreatic beta cells may be isolated and thereafter cultured in an appropriate culture medium to expand the cells for use in organ transplantation.

EXAMPLE 6

By a process similar to that of Example 1, making use of an appropriate monoclonal antibody, specific populations of cytotoxic T-lymphocytes may be selected, such as tetanus-toxoid primed lymphocyte cells. These cells can be separated by an analogous technique similar to that of Example 1, manipulated in vitro and transfused to effect targeting of the cytotoxic cells to specific B-lymphocytes which mediate autoimmune disease (for example, Myasthenia Gravis). In this way, the disease mediating cells could be destroyed in situ.

WHAT IS CLAIMED IS:

1. A system for the selective separation of a specific cell population from a heterogeneous cell mixture comprising:

means for obtaining a selective cell concentrate from said heterogeneous cell mixture by separating said selective cell concentrate based upon the physical properties of said concentrate;

a container having particle means, said particle means having attached thereto a substance capable of binding to some cells only of said cell concentrate;

means for intimately contacting said cell concentrate with said particle means within said container, to thereby permit incubation of said cell concentrate with said particle means, to thereby selectively bind a specific cell population from said cell concentrate to said particle means, creating a particle/cell conjugate; and

means for separating said particle/cell conjugate from the remainder of said cell concentrate.

2. The system of Claim 1, wherein the initial cell concentrate fluid volume remains within said container during the incubation period.

3. The system of Claim 1, further comprising means for introducing additional volume of said cell concentrate into said container during the incubation period.

4. The system of Claim 1, in which said particle means comprises a paramagnetic material, and the means for separating said particle/cell conjugate comprises magnet means for causing said particle/cell conjugate to be retained in a fixed location as remaining, unbound portions of said cell concentrate are removed from said location.

5. The system of Claim 4, in which said magnet means defines first and second spaced magnet members, said second magnet member provides a shallower magnetic field but a stronger magnetic holding force at its surface than the first magnet member.

6. The system of Claim 4, in which said magnet means is positioned adjacent means for carrying and positioning at least one flexible, collapsible container to permit at least one inner wall portion of said container to be within the magnetic field of said magnet means, said inner wall portion serving as said fixed location.

7. The system of Claim 6, in which flat-pressing means are also provided to press said collapsible container flat while said inner wall portion is within said magnetic field of provide a cell flow path thickness of 0.02 to 0.1 inch therein, to facilitate separation of said particle/cell conjugate from the remainder of said cell concentrate.

8. The system of Claim 4, in which said container having particle means is a flexible, collapsible container.

9. The system of Claim 4, in which said container having particle means is aseptically connected to a flexible, multiple-chamber insert means for a blood cell separation centrifuge, whereby a concentrated fraction of freshly collected blood cells may be aseptically transferred from said insert means to said container having particle means without need of forming a sterile connection therebetween.

10. For use in the system as in Claim 4, a first flexible, collapsible container which sealingly contains said paramagnetic particle means, said flexible, collapsible

container being aseptically connected to further flexible, collapsible container means for receiving processed cells from said first container, said first container being also aseptically connected to a flexible means for a blood cell separation centrifuge whereby a freshly concentrated fraction of collected blood cells may be aseptically transferred from the insert means to said first container and from said first container to the further container means without the need of forming a sterile connection therebetween.

11. The system of Claim 10, in which a second flexible, collapsible container is sealingly aseptically connected to and positioned between said first container and said further collapsible container means, for interaction with magnet means for added retention of said paramagnetic particle means during all separation.

12. The system of Claim 4 in which said substance which is attached to the particle means is selected from the group consisting of antibodies, antigens, proteins, glycoproteins, polysaccharides, lipopolysaccharides, nucleic acids, and lipids.

13. The system of Claim 4 in which a flow path for said cell concentrate is provided which comprises an enlarged, flexible container defining inlet and outlet ports at opposed ends thereof, the cross sectional area of said inlet and outlet ports being no more than one-quarter of the cross sectional area of said container.

14. The system of Claim 13 in which said container is of hexagonal shape, said inlet and outlet ports being positioned at opposed corners thereof.

15. A system for the selective separation of the specific cell population from a heterogeneous cell mixture comprising:

a first flexible collapsible container having particle means, said particle means having attached to said particle means a substance capable of binding to the desired cells only, to form a particle/cell conjugate;

means defining a flow path including said flexible, collapsible container;

means for intimately contacting said cell mixture with the particle means within said container, to thereby permit incubation of said cell mixture with said particle means, to thereby selectively bind the specific cell pop7population from said cell mixture to said particle means, to create said particle/cell conjugate; and

first and second spaced magnet members located sequentially along said flow path, for causing said particle/cell conjugate to be retained in a fixed location as remaining, unbound portions of said cell mixture are moved along said flow path to be removed from said location; said first and second magnet members being positioned adjacent means for carrying and positioning flexible, collapsible container means to permit an inner wall portion of said container means to be within the magnetic field of said magnet means, said inner wall portion serving as said fixed location, said second magnet member being positioned downstream from said first magnet member and providing a shallower magnetic field, but a stronger magnetic holding force at its surface than the first magnet member.

16. The system of Claim 15 in which a second flexible, collapsible container is sealingly aseptically connected to said first container having particle means, said first magnet member being positioned against said first flexible container and said second magnet member being positioned against said second flexible, collapsible container.

17. The system of Claim 16 in which the second flexible container positioned against the second magnet means defines inlet ports at opposed ends thereof, the cross sectional area of said inlet and outlet ports being no more than one-fourth of the cross sectional area of said second flexible container.

18. The system of Claim 17 in which said second container is of hexagonal shape, said inlet and outlet ports being positioned at opposed corners thereof.

19. The system of Claim 17, in which flat-pressing means are also provided, to press said second flexible collapsible container flat while said inner wall portion is within said magnetic field to provide a cell flow path thickness of 0.02 to 0.1 inch therein to facilitate separation of the particle/cell conjugate from the remainder of said cell mixture.

20. The system of Claim 17, in which said container having particle means is aseptically connected to a flexible, mutliple;-chamber insert means for a blood cel separation centrifuge, whereby a concentrated fraction of freshly collected blood cells may be aseptically transferred from said insert means to said container having particle means without the need of forming a sterile connection therebetween.

21. The system of Claim 20, in which further collapsible container means are connected along said flow path to said first flexible, collapsible container having particle means, said second flexible, collapsible container is sealingly, aseptically connected to and positioned between said container having particle means and said further collapsible container means.

22. A method for the selective separation of blood cells which comprises the steps of:

operating a blood cell centrifuge to collect blood cells in a first container; sealing said first container and disconnecting said first container from the centrifuge; mixing the blood cells placed in said first container with paramagnetic particle means in said first container having attached to said particle means a substance capable of binding to some of said desired cells only; opening a connection of said first container to the inlet of a cell separation set including tubing and containers; installing said first container and a secondary separation container of said separation set which is connected to said first container in magnetic separator means; initiating flow through said first container and secondary container with both said first and second containers being under the influence of said magnetic separator means to immobilize said particle means and cells bound thereto; opening flow from said second container to first storage container means to receive cells not bound to said particle means; closing flow between said second container and said first storage container means; removing said first and second containers from the magnetic separation means; opening flow between said second container and second storage container means, and causing flow of cells bound to said particle means into said second storage container means to provide separation of said bound cells from remaining cells in the system.

23. A method for the selective separation of cells from a mixture of cells which comprises the steps of:

placing said mixture of cells in a first container having paramagnetic particle means therein, said particle means having attached thereto a substance capable of binding to some of said cells only; opening a connection of said first container to the inlet of a cell separation set including tubing and containers; installing said first container and a second separation container of said separation set, which is connected to said

first container, in magnetic separator means having first and second magnets, said first magnet being positioned against said first container and said second magnet being positioned against said second container, said second magnet providing a shallower magnetic field but a stronger magnetic holding force at its surface than the first magnet, with said first magnet having a magneticfield reach substantially equivalent to the width of said first container; initiating flow through said first container and second container with both said first and second containers being under the influence of said magnetic separator means to immobilize said particle means and cells bound thereto; and causing flow from said second container to first storage container means to receive cells not bound to said particle means.

24. The method of Claim 23 which includes the added steps of closing flow between said second container and said first storage container means; removing said first and second container from the magnetic separation means; opening flow between the second container and second storage container means, and causing t flow of cells separated by binding to said particle means into said second storage container means to provide separation hereof from remaining cells in the system.

25. The method of Claim 23 in which the cell flow path through at least said second container under the influence of said magnetic separator means is maintained at a thickness of 0.02 to 0.1 inch.

26. The method of Claim 23 in which the second container defines an inlet and an outlet on opposed ends thereof for said cell flow path, said inlet and outlet having a cross sectional area of no more than one-fourth the cross sectional area of said second container.

27. The method of Claim 26 in which said second container is of hexagonal shape, said inlet and outlet being positioned at opposed corners thereof.

28. The method of Claim 23 which includes the added steps of closing flow between said second container and the first storage container means; causing separation of the connection between those cells which are bound to the paramagnetic particle means; causing flow of said cells from said first and second containers into second storage container means while the first and second containers are under the influence of said magnetic separator means whereby said particle means are prevented from flowing with said cells into the second storage container means, for separation of the cells from the particle means.

29. The method of Claim 23 in which said paramagnetic particle means carry on their surfaces a specific antibody to cells selected from the group consisting of B-lymphoma, neuroblastoma, breast cancer, leukemia, T-lymphocytes, and pluripotent stem cells.

30. The method of Claim 29 in which said mixture of cells placed in the first container having paramagnetic particle means is a preparation of mononuclear cells previously separated from bone marrow.

31. The method of Claim 23 in which said paramagnetic particle means are coated with a specific antibody to cells selected from the group consisting of liver hepatocytes and insulin secreting pancreatic beta cells, for separation thereof from a larger group of cells.

32. The method of Claim 23 in which said paramagnetic particle means carry on their surfaces a specific antibody to a

specific population of cytotoxic T-lymphocytes, for separation thereof from a mixture of cells.

33. The system of claim 16 wherein said first magnetic means possess a magnetic field having a reach substantially equivalent to the width of said first flexible, collapsible container.

34 The system of claim 33 wherein said first magnetic means possesses a magnetic field having a reach of from about one-half an inch to about one inch.

35 The system of claim 33 wherein said first magnetic means possesses a magnetic field having a reach of from about one-half to about three-quarters of an inch.

36 The method of claim 23 wherein said first magnet possesses a magnetic field having a reach of from about one-half an inch to about one inch.

37 The method of claim 23 wherein said first magnet possesses a magnetic field having a reach of from about one-half to about three-quarters of an inch.

38 The method of claim 31 wherein said first magnet possesses a magnetic field having a reach of from about one-half an inch to about one inch.

39 The method of claim 31 wherein said first magnet possesses a magnetic field having a reach of from about one-half to about three-quarters of an inch.

AMENDED CLAIMS

[received by the International Bureau
on 13 March 1990 (13.03.90);
original claims 1, 4 and 5 replaced
by amended claim 1; original claims 6, 8, 9,
10, 12 and 13 amended; remaining claims unchanged
(3 pages)]

1. A system for the selective separation of a specific cell population from a heterogeneous cell mixture comprising:

means for obtaining a selective cell concentrate from said heterogeneous cell mixture by separating said selective cell concentrate based upon the physical properties of said concentrate;

a container having particle means formed from a paramagnetic material, said particle means having attached thereto a substance capable of binding to some cells only of said cell concentrate;

means for intimately contacting said cell concentrate with said particle means within said container, to thereby permit incubation of said cell concentrate with said particle means, to thereby selectively bind a specific cell population from said cell concentrate to said particle means, creating a particle/cell conjugate; and

magnetic means for separating said particle/cell conjugate, said magnet means causing said particle/cell conjugate to be retained in a fixed location as remaining, unbound portions of said cell concentrate are removed from said location, said magnet means defines first and second spaced magnet members, said second magnet member provides a shallower magnetic field but a stronger magnetic holding force at its surface than the first magnet member.

2. The system of Claim 1, wherein the initial cell concentrate fluid volume remains within said container during the incubation period.

3. The system of Claim 1, further comprising means for introducing additional volume of said cell concentrate into said container during the incubation period.

4. CANCELED

5. CANCELED

6. The system of Claim 1, in which said magnet means is positioned adjacent means for carrying and positioning at least one flexible, collapsible container to permit at least one inner wall portion of said container to be within the magnetic field of said magnet means, said inner wall portion serving as said fixed location.

7. The system of Claim 6, in which flat-pressing means are also provided to press said collapsible container flat while said inner wall portion is within said magnetic field of provide a cell flow path thickness of 0.02 to 0.1 inch therein, to facilitate separation of said particle/cell conjugate from the remainder of said cell concentrate.

8. The system of Claim 1, in which said container having particle means is a flexible, collapsible container.

9. The system of Claim 1, in which said container having particle means is aseptically connected to a flexible, multiple-chamber insert means for a blood cell separation centrifuge, whereby a concentrated fraction of freshly collected blood cells may be aseptically transferred from said insert means to said container having particle means without need of forming a sterile connection therebetween.

10. For use in the system as in Claim 1, a first flexible, collapsible container which sealingly contains said paramagnetic particle means, said flexible, collapsible

container being aseptically connected to further flexible, collapsible container means for receiving processed cells from said first container, said first container being also aseptically connected to a flexible means for a blood cell separation centrifuge whereby a freshly concentrated fraction of collected blood cells may be aseptically transferred from the insert means to said first container and from said first container to the further container means without the need of forming a sterile connection therebetween.

11. The system of Claim 10, in which a second flexible, collapsible container is sealingly aseptically connected to and positioned between said first container and said further collapsible container means, for interaction with magnet means for added retention of said paramagnetic particle means during all separation.

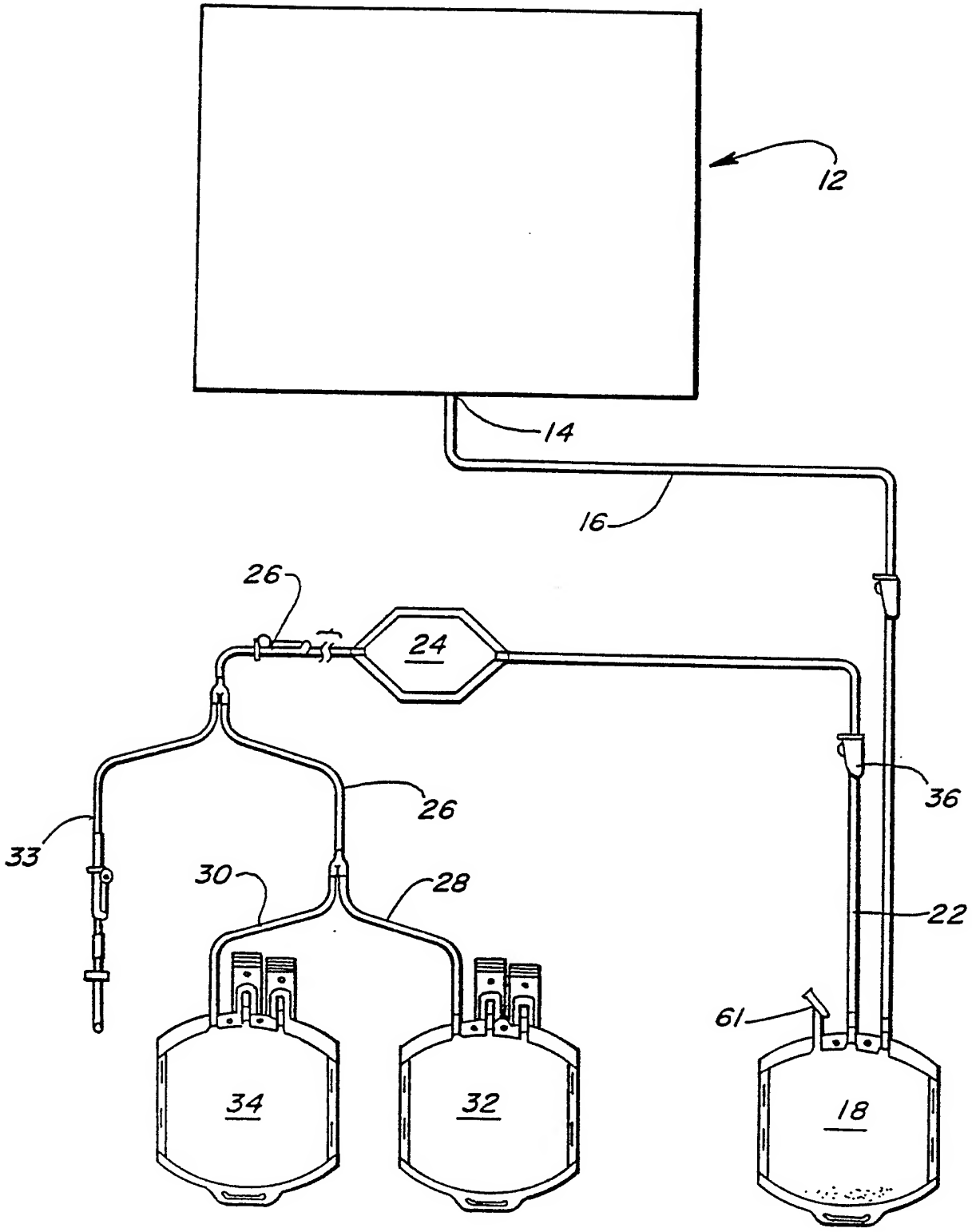
12. The system of Claim 4 in which said substance which is attached to the particle means is selected from the group consisting of antibodies, antigens, proteins, glycoproteins, polysaccharides, lipopolysaccharides, nucleic acids, and lipids.

13. The system of Claim 1 in which a flow path for said cell concentrate is provided which comprises an enlarged, flexible container defining inlet and outlet ports at opposed ends thereof, the cross sectional area of said inlet and outlet ports being no more than one-quarter of the cross sectional area of said container.

14. The system of Claim 13 in which said container is of hexagonal shape, said inlet and outlet ports being positioned at opposed corners thereof.

15. A system for the selective separation of the specific cell population from a heterogeneous cell mixture comprising:

FIG. 1



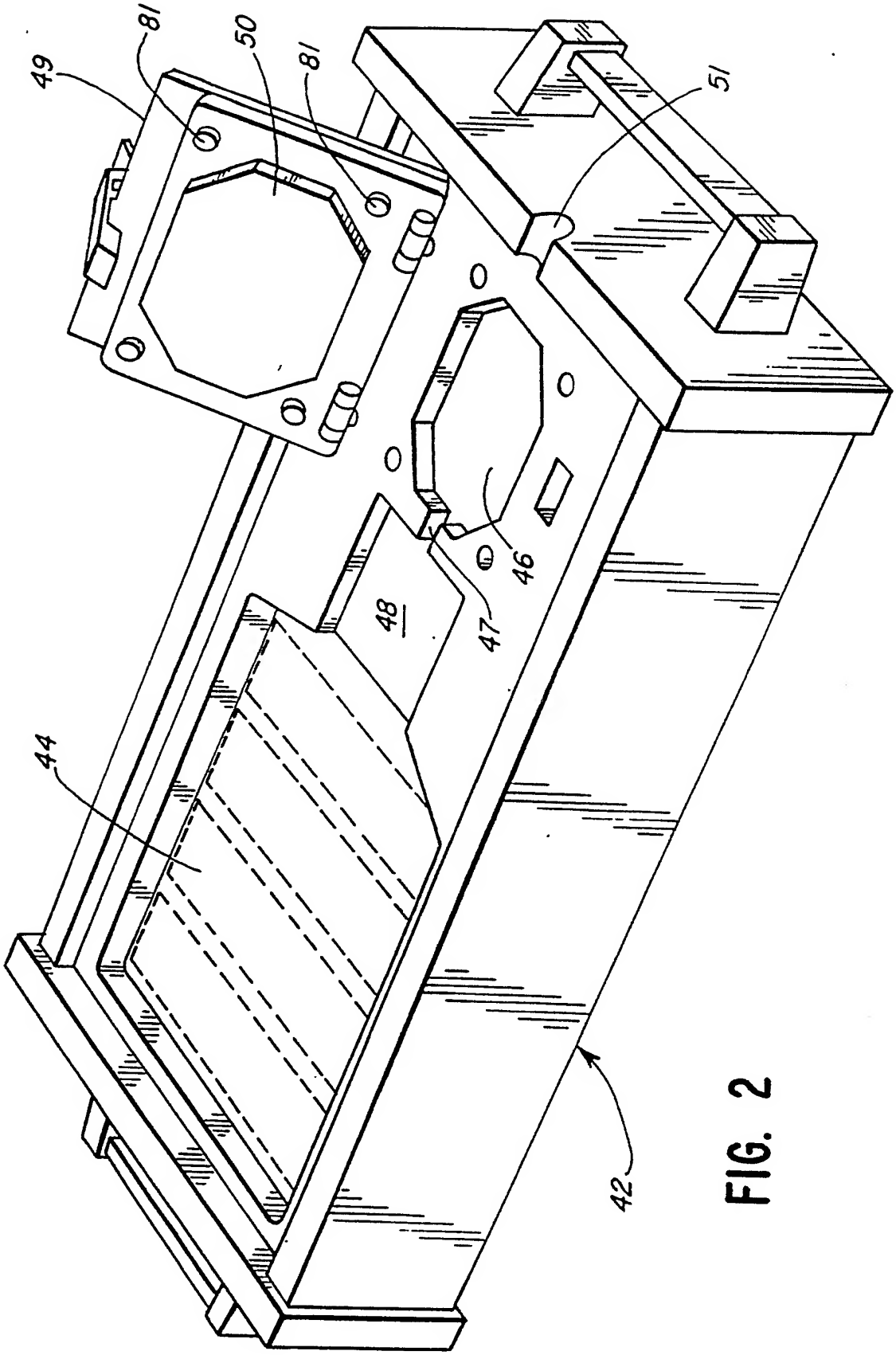


FIG. 2

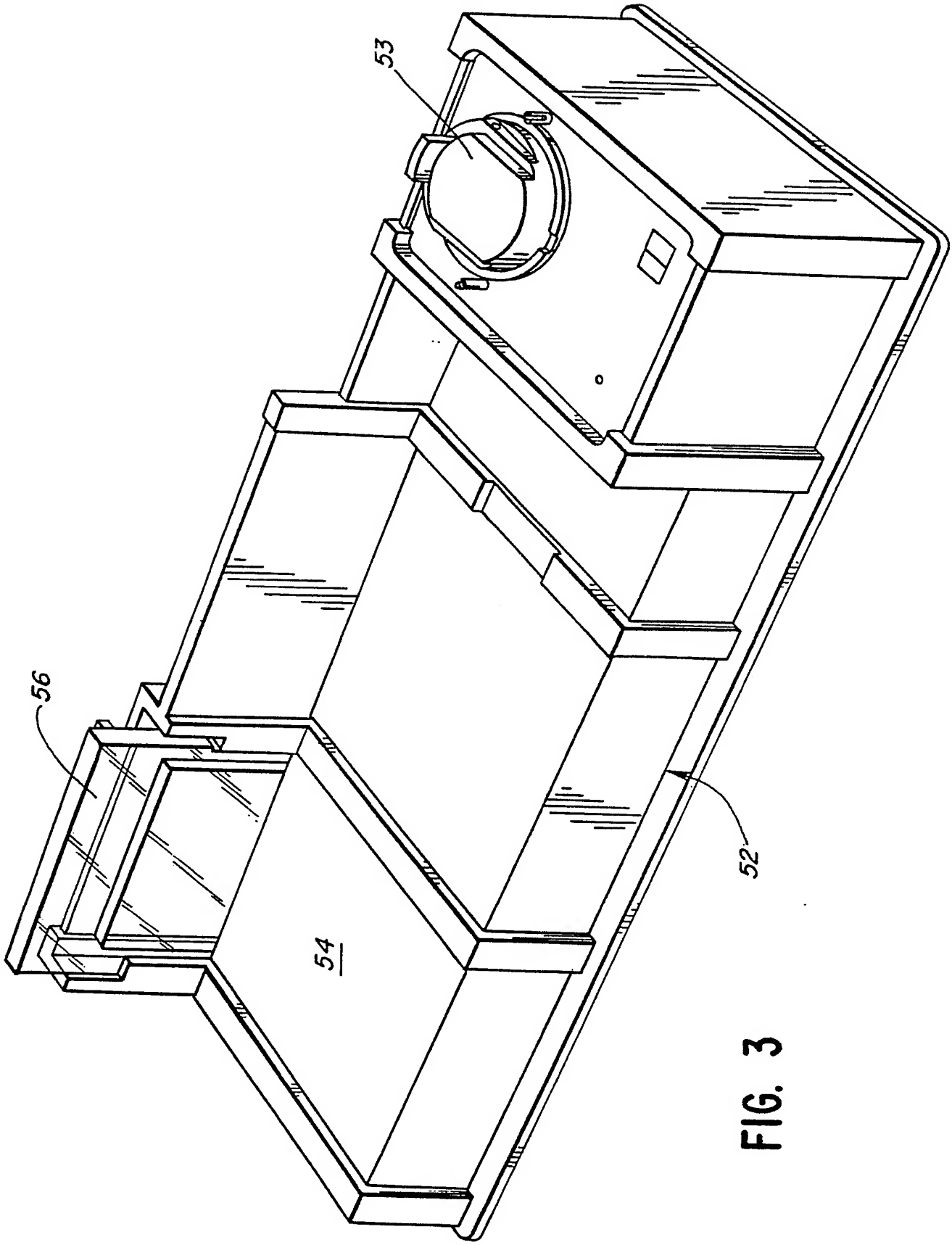


FIG. 3

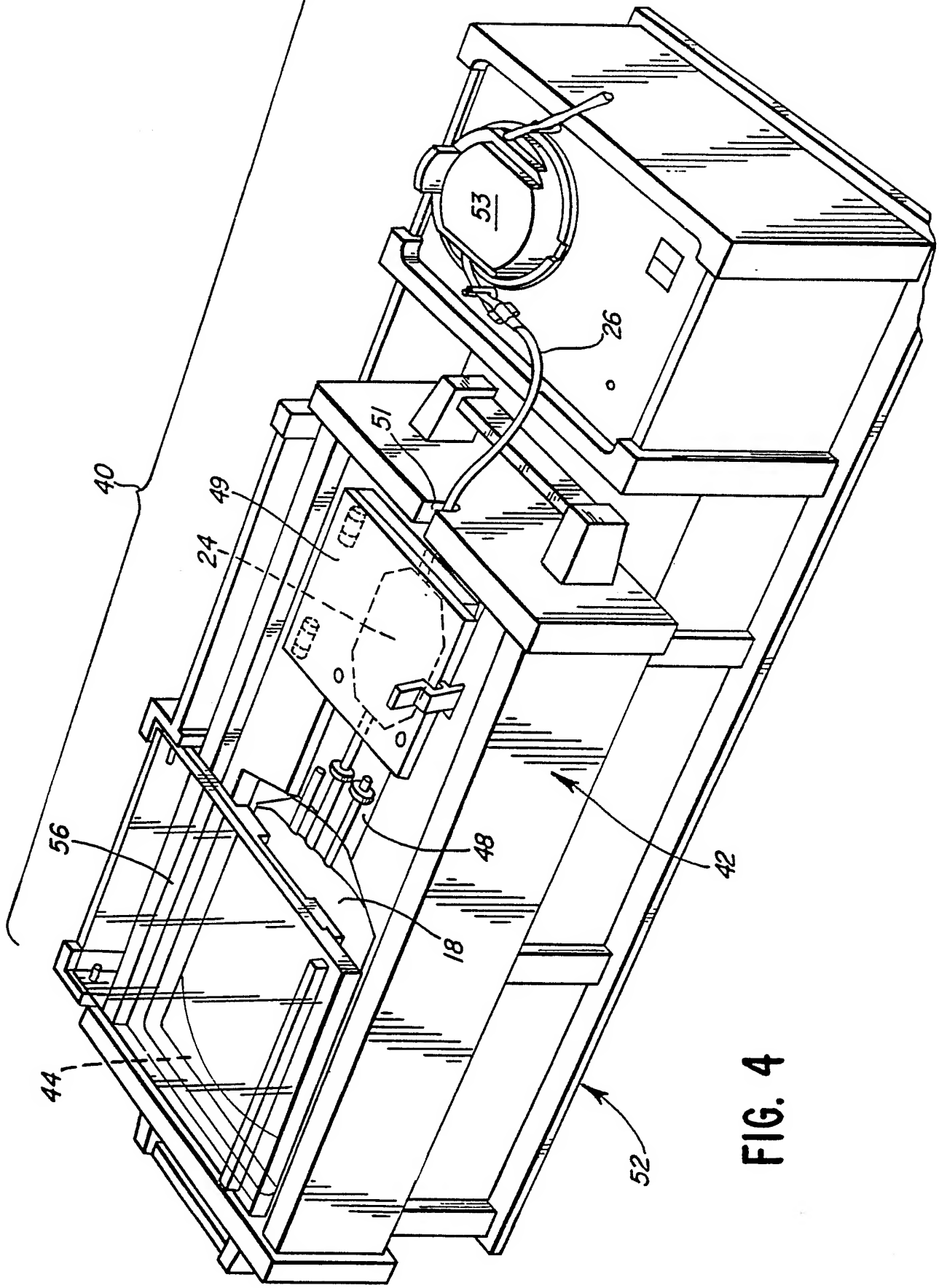


FIG. 4

FIG. 5

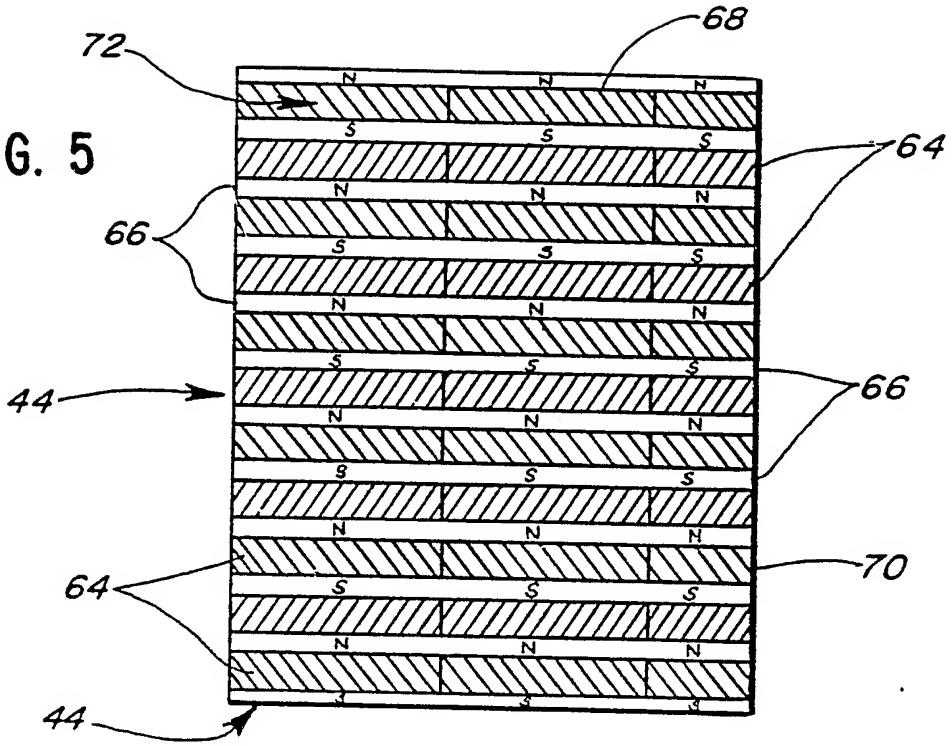


FIG. 6

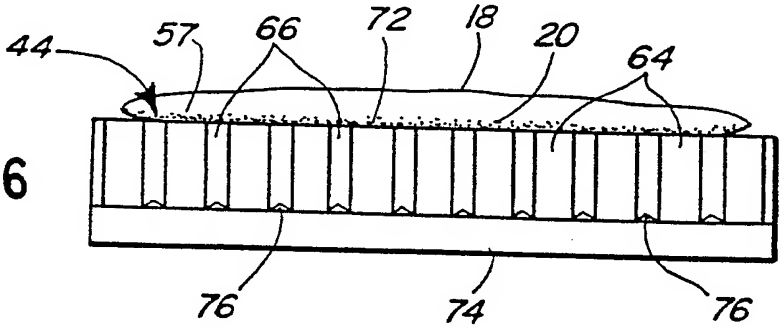
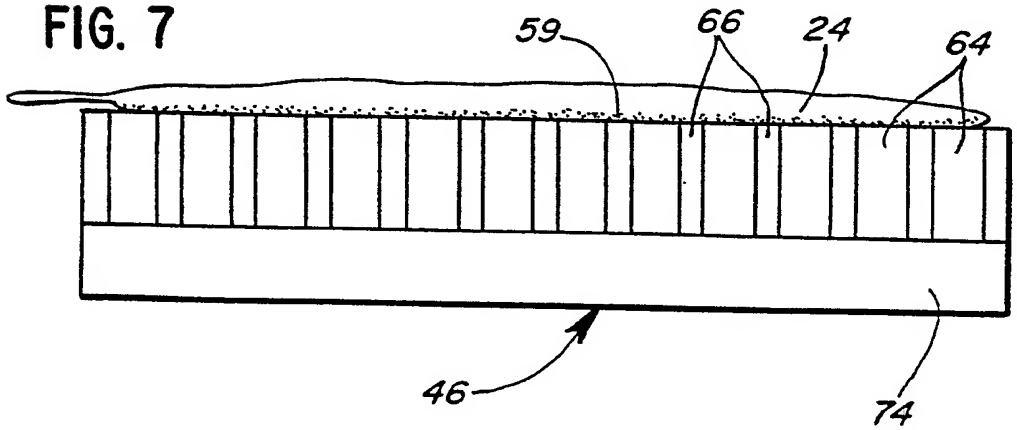


FIG. 7



INTERNATIONAL SEARCH REPORT

International Application No PCT/US 89/04527

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: C 12 N 5/00, C 12 M 3/00, B 03 C 1/02, A 61 M 1/36
G 01 N 3/543, 553, 48, A 61 K 39/395

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC5	A 61 M; B 03 C; C 12 N; C 12 M; G 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X,Y	US, A, 4710472 (J.W. SAUR ET AL.) 1 December 1987, see the whole document	1-4,6-8, 12,22
A	--	5,15
Y	EP, A2, 0184710 (BAYER AG) 18 June 1986, see pages 14-15	1,2,4, 12,22
	--	
A	WO, A1, 88/06632 (ADVANCED MAGNETICS, INC.) 7 September 1988, see page 14, lines 28-33, page 5, lines 9-29 and pages 31-33	4
	--	

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
15th February 1990	27.02.90
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	T.K. WILLIS

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	WO, A1, 89/06280 (E.I. DU PONT DE NEMOURS AND COMPANY) 13 July 1989, see the whole document	1,23
A	WO, A1, 83/02405 (THOMAS A. REED) 21 July 1983, see the whole document	1,23
A	WO, A1, 87/06844 (OMEGA MEDICINTEKNIK AB) 19 November 1987, see the whole document	20

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/US 89/04527

SA 32164

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 08/11/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A- 4710472	01/12/87	NONE	
EP-A2- 0184710	18/06/86	DE-A- 3444939	12/06/86
		JP-A- 61140523	27/06/86
WO-A1- 88/06632	07/09/88	NONE	
WO-A1- 89/06280	13/07/89	EP-A- 0323829	12/07/89
WO-A1- 83/02405	21/07/83	DE-A-C- 3200988	28/07/83
		EP-A-B- 0098286	18/01/84
		DE-A- 3373789	29/10/87
		US-A- 4855045	08/08/89
WO-A1- 87/06844	19/11/87	WO-A- 87/06857	19/11/87
		SE-A- 8602242	17/11/87
		SE-A- 8605456	17/11/87
		AU-D- 73997/87	01/12/87
		AU-D- 74000/87	01/12/87
		EP-A- 0304431	01/03/89
		EP-A- 0305397	08/03/89

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82